Journal of Medical Virology 58:76–78 (1999)

Analysis of Strain Variation of R1 Repeated Structure in Varicella-Zoster Virus DNA by Polymerase Chain Reaction

Masami Yoshida,[1]* Takahiro Tamura,[1] and Masataro Hiruma[2]

[1]*Department of Dermatology, Kinki University School of Medicine, Osaka, Japan*
[2]*Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan*

The tandem direct reiteration R1 in the varicella-zoster virus genome consists of two elements: units composed of 18 bp and those having 15 bp, both of whose numbers and types of combination patterns vary among strains. The variations of the R1 structure were examined in order to differentiate between the wild strains and a varicella vaccine (Oka) strain by the polymerase chain reaction using two primer sets. The results showed that the 31 wild strains were classified into nine patterns: the R1 structure consisted of a combination of 5 to 7 18-bp units and 8 to 12 15-bp units. The strain with a combination of 6 18-bp units and 10 15-bp units was found to be predominant in Japan, and the same pattern was found in the vaccine strain, so that differentiation between the vaccine strain and the wild strains in Japan merely by analysis of R1 in VZV genome is difficult. ***J. Med. Virol. 58:76–78, 1999.***

KEY WORDS: R1; repeating sequence; strain variation; varicella-zoster virus

INTRODUCTION

Five tandem direct reiterations, R1–R5, are present on the varicella-zoster virus (VZV) genome (Fig. 1) [Straus et al., 1983; Davison and Scott, 1986; Hondo et al., 1987; Kinoshita et al., 1988]. The numbers and patterns of combination of the repeating units vary among strains [Davison and Scott, 1986; Kinchington et al., 1986; Casey et al., 1988; Kinoshita et al., 1988; Hondo and Yogo, 1988]. Recently, variations of the numbers of repetitions of the units in three regions, R2, R4, and R5, between different clinical strains have been reported on the basis of analysis using the polymerase chain reaction (PCR) [Takada et al., 1995]. On the other hand, some difficulty remains in comparing differences of R1 between VZV strains, because R1 consists of two types of repeating units, an 18-bp unit and a 15-bp unit, which vary in number and in patterns of combination from strain to strain (Fig. 2) [Kinoshita et al., 1988]. However, if analysis of these patterns in the R1 region is possible by PCR, it will be useful for the differentiation of VZV strains, especially between the wild strains and a vaccine (Oka) strain. The intratypic variations of R1 by PCR using two pairs of primer sets are described.

MATERIALS AND METHODS

The 31 specimens used in this study were taken from patients with herpes zoster. All specimens were derived from different patients; 20 of the patients lived in Osaka, and 11 in Tokyo. The specimens were obtained from vesicles or pustules by rubbing with sterilized cotton swabs, which were then washed into phosphate-buffered saline. A varicella vaccine (Oka) strain was also analyzed.

Each specimen was treated with Gene Releaser (Bioventures, Murfreesboro, TN) according to the manufacturer's instructions. Briefly, 20 μl of Gene Releaser was added to 1 μl of specimen in a PCR tube, and then the following thermocycle program was performed: 65°C for 30 sec, 8°C for 30 sec, 65°C for 90 sec, 97°C for 180 sec, 8°C for 60 sec, 65°C for 180 sec, 97°C for 60 sec, 65°C for 60 sec, and 80°C for a few min.

The standard 100-μl PCR mixtures contained 2.5 units of Takara-Taq DNA polymerase, each deoxynucleoside triphosphate at a concentration of 200 μM, 10 μl of reaction buffer (500-mM KCl, 100-mM Tris-HCl, pH 8.3, 15-mM $MgCl_2$, 0.01% gelatin) (TaKaRa, Kyoto, Japan), 1 μl of each primer (0.1 μM), and 10 μl of template. This was overlaid with mineral oil. The amplication of VZV DNA was carried out in Cetus thermal cycler 480 (Perkin-Elmer, Norwalk, CT) by the method of Saiki et al., [1988].

The sequence information for designing the primers for PCR was taken from the reports [Davison and Scott,

*Correspondence to: Dr. Masami Yoshida, Department of Dermatology, School of Medicine, Kinki University, 377-2 Ohnohigashi, Osakasayama-shi, Osaka, 589-8511, Japan.

Accepted 26 October 1998

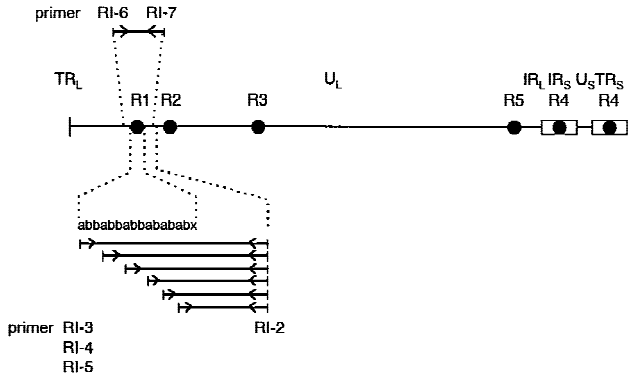

Fig. 1. Arrangement of VZV genome and locations of primer sets in this study.

Sequences of repeating units of RI*
Structure of RI*

a	: GGACGCGATCGACGACGA	18 bp
a'	: GGACGCGATTGACGACGA	18 bp
a"	: GGACGCGATCGACGACAA	18 bp
b	: GGGAGAGGCGGAGGA	15 bp
b'	: GGACGCGGCGGAGGA	15 bp
χ	: GGA	3 bp

For example:
H-S1 strain: abbabbabbababab χ

*Sequence information was taken from Davison et al. and Kinoshita et al.

Fig. 2. Structure of R1 and sequences of repeating units.

1986; Kinoshita et al., 1988]. First, three kinds of sense primers, named R1-3, R1-4, and R1-5, were designed to correspond to the three 18-bp units [Kinoshita et al., 1988], because it has been reported that two-point mutations exist in each 18-bp unit [Kinoshita et al., 1988]. Primer R1-3 corresponds to an 18-bp unit consisting of the nucleotide sequence a, R1-4 to that with sequence a′, and R1-5 to that with sequence a″ (Figs. 1 and 2). The common antisense primer of these sets, named R1-2, was designed at a location separated by 264 bp from the R1 region (Fig. 1) [Davison and Scott, 1986].

Using these primer pairs, multiple bands corresponding to the numbers of 18-bp units could possibly be detected. The distance between two bands of 18-bp units in the electrophoresis pattern allowed the number of repetitions of 15-bp units to be estimated. Another primer set, named R1-6 and R1-7, was designed at the locations flanking the R1 region (Fig. 1). This set amplified a single band whose size was calculated as 38 + [(18 × number of unit repetitions) + (15 × number of unit repetitions) + 3] + 34 bp (Figs. 1 and 2). Therefore, the total number of 18-bp and 15-bp units was verified from the size of a single band using the set R1-6 and R1-7. The sequences of these primers are as follows: R1-2, 5′-TCGTATAATCCTTGGACCGTA-3′; R1-3, 5′-GGACGCGATCGACGACGA-3′; R1-4, 5′-GGACGCGATTGACGACGA-3′; R1-5, 5′-GGACGCGATCGACGACAA-3′; R1-6, 5′-ACGGAGAGGGAAGCTAATGAG-3′; and R1-7, 5′-GTCTCGACTGCAAACTTGACT-3′.

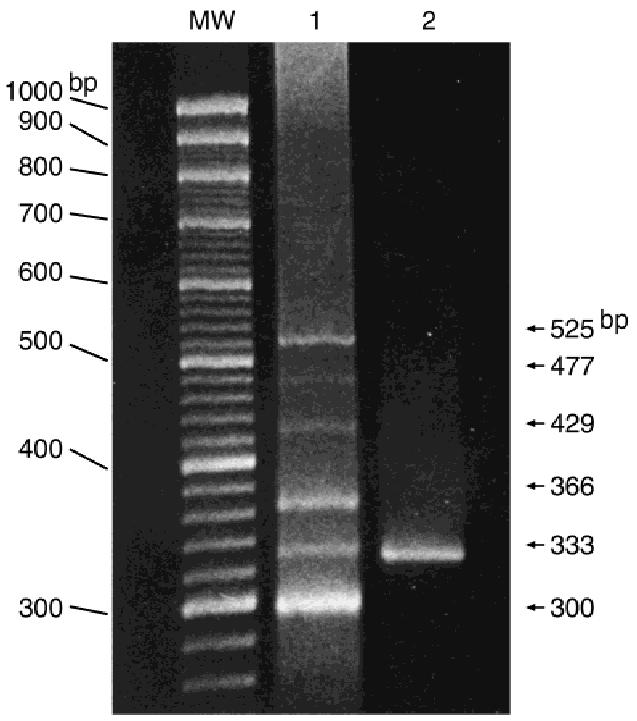

Fig. 3. Electrophoresis pattern of varicella vaccine (Oka) strain. Lane 1: 18-bp unit elements in R1, showing the J1 pattern; lane 2: single-band containing R1. MW: molecular weight marker.

Using the three primer sets R1-3 and R1-2, R1-4 and R1-2, and R1-5 and R1-2, denaturation was carried out at 95°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 2 min. On the other hand, using the primer set R1-6 and R1-7, thermal cycling was performed at 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min. After 30 cycles of PCR, the reaction products were electrophoresed in 3% NuSieve 3:1 agarose gel (FMC BioProducts, Vallensbaek Strand, Denmark) with Superladder-low (GenSura, Del Mar, CA) used as a size marker, and then stained with ethidium bromide. Photographs were taken on the UV illuminator and then analyzed.

RESULTS

The electrophoresis patterns of PCR products of the vaccine strain using two pairs of primer sets are shown in Figure 3. The pattern analysis showed that the vaccine strain had a combination of 6 18-bp units and 10 15-bp units. When the 31 wild strains were analyzed by the same method, the R1 structure had a combination of 5 to 7 18-bp units and 8 to 12 15-bp units (Fig. 4). The basic structure of R1 consisted of a repeated pattern of units in which two 15-bp units appeared between single 18-bp units in the left portion of R1, and, in the right portion, 15-bp units and 18-bp units alternated. The strains were classified into two major groups on

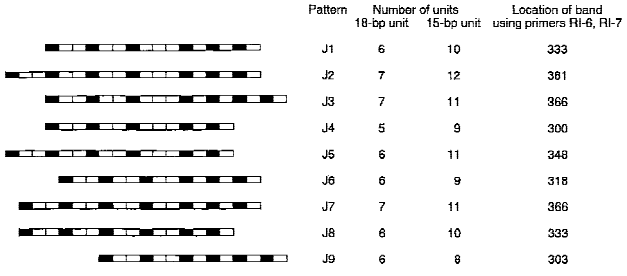

Fig. 4. Comparison of R1 structures in varicella-zoster virus strains.

the basis of their structures, one group having a single sequence of three 15-bp units flanked by single 18-bp units in R1, and the other group lacking this structure. The former group comprised five patterns, and the latter four, and the patterns were named J1–J9. In comparison with the size differences of single PCR bands using merely R1-6 and R1-7 primers, both J1 and J8 had combinations of 6 18-bp and 10 15-bp units, so that J1 (333 bp) and J8 (333 bp) could not be distinguished; nor could J3 (366 bp) and J7 (366 bp), which consisted of 7 18-bp and 11 15-bp units, respectively. J4 (300 bp) and J9 (303 bp) could not be distinguished from the R1-6 and R1-7 primers because their size difference is small (Fig. 4).

Table I compares the strains obtained from two major Japanese cities, Osaka and Tokyo. The J1 pattern was found in 8 of 20 in Osaka and in 4 of 11 in Tokyo. It was also found that the varicella vaccine (Oka) strain established in Japan had the J1 pattern (Fig. 3).

TABLE I. Comparison of Varicella-Zoster Virus Strains Obtained From Osaka and Tokyo, Japan

	Number	
Pattern	Osaka	Tokyo
Group A		
J1	8	4
J2	2	0
J3	2	0
J4	2	4
J5	2	0
Group B		
J6	2	2
J7	0	1
J8	1	0
J9	1	0

DISCUSSION

It has been reported that analysis of the endonuclease digestion patterns of VZV DNA shows the size differences of the R1-containing fragments to be small or undetectable between VZV strains. Because the R1 structure is constituted of a combination of 18-bp and 15-bp units whose numbers vary among VZV strains, small differences in size are not detectable in electrophoresis either [Kinoshita et al., 1988].

In this study, 31 VZV strains were classified into nine types on the basis of an examination of the two types of units. Moreover, by PCR, minor size differences of single bands obtained in electrophoresis were detectable in all but a few patterns. However, the differentiation between the strains with J1 and J8 patterns (both having 6 units of 18 bp and 10 of 15 bp), those with J3 and J7 patterns (both with 7 and 11 of the respective units), and those with J4 (300 bp) and J9 (303 bp) patterns was impossible. Therefore, it is stressed that pattern analysis of the combination of 18-bp and 15-bp units is necessary, when epidemiologically unrelated VZV strains are differentiated by analysis of the R1 region in the VZV genome.

The strains with the J1 pattern (6 18-bp and 10 15-bp units) were predominant in both Osaka and Tokyo, suggesting that this type of strains may be predominant in most of Japan. The varicella vaccine (Oka) strain also had the same pattern. These results suggest that differentiation between the vaccine strain and wild strains in Japan is difficult by mere analysis of the R1 in the VZV genome. On the other hand, it has been reported that numbers of repeating units in R2, R5, and *PstI* cleavage site mutation between K and N segments are useful markers for differentiation between the vaccine strain and wild strains in Japan [Hayakawa et al., 1984; Hondo et al., 1989; Takada et al., 1995]. This study shows that approximately half of the wild strains in Japan have different R1 structures from that of the caccine strain, suggesting that when pattern analysis of R1 is used in addition to these markers, differentiation between the vaccine strain and the wild strains will be more accurate.

REFERENCES

Casey TA, Ruyechan WT, Flora MN, Reinhold W, Straus SE, Hay J. 1985. Fine mapping and sequencing of a variable segment in the inverted repeat region of varicella-zoster virus DNA. J Virol 54:639–642.

Davison AJ, Scott JE. 1986. The complete DNA sequence of varicella-zoster virus. J Gen Virol 67:1759–1816.

Hayakawa Y, Torigoe S, Shiraki K, Yamanishi K, Takahashi M. 1984. Biologic and biophysical markers of a live varicella vaccine strain (Oka): identification of clinical isolates from vaccine recipients. J Inf Dis 149:956–963.

Hondo R, Yogo Y, Kurata T, Aoyama Y. 1987. Genome variation among varicella-zoster virus isolates derived from different individuals and from the same individuals. Arch Virol 93:1–12.

Hondo R, Yogo Y. 1988. Strain variation of R5 direct repeats in the right-hand portion of the long unique segment of varicella-zoster virus DNA. J Virol 62:2916–2921.

Hondo R, Yogo Y, Yoshida M, Fujima A, Itoh S. 1989. Distribution of varicella-zoster virus strains carrying a PstI-site less mutation in Japan and DNA change responsible for the mutation. Jpn J Exp Med 59:233–237.

Kinchington PR, Remenick J, Ostrove JM, Straus SE, Ruyechan WT, Hay J. 1986. Putative glycoprotein gene of varicella-zoster virus with variable copy numbers of a 42-base-pair repeat sequence has homology to herpes simplex virus glycoprotein C. J Virol 59:660–668.

Kinoshita H, Hondo R, Taguchi F, Yogo Y. 1988. Variation of R1 repeated sequence present in open reading frame 11 of varicella-zoster virus strains. J Virol 62:1097–1100.

Saiki RK, Gelfand DH, Stoffel S, Scharf S, Scharf SJ, Higuchi RG, Horn GT, Mullis KB, Erlish HA. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Straus SE, Hay J, Smith H, Owens J. 1983. Genome differences among varicella-zoster virus isolates. J Gen Virol 64:1031–1041.

Takada M, Suzutani T, Yoshida I, Matoba M, Azuma M. 1995. Identification of varicella-zoster virus strains by PCR analysis of three repeat elements and a PstI-site-less region. Clin Microbiol 33:658–660.